Seminars in Cell & Developmental Biology 20 (2009) 55–62

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

# Biosensors for cancer markers diagnosis

Ibtisam E. Tothill*

*Cranfield Health, Cranfield University, Cranfield, Bedfordshire, MK 43 0AL England, UK*

ARTICLE INFO

*Article history:*
Available online 4 February 2009

*Keywords:*
Cancer markers
Biosensors
Nanomaterials
Diagnostics
Point-of-care devices

ABSTRACT

Point-of-care diagnostic devices present a viable option for the rapid and sensitive detection and analysis of cancer markers. With the growing number of cancer cases being diagnosed worldwide and the increased number of fatalities due to late disease detection, biosensors can play an important role in the early diagnosis of cancer. Molecular profiles of patients are being increasingly studied using new molecular tools such as genomic and proteomic techniques. These methods combined with bioinformatics tools are generating new data which is being employed in the elucidation of new disease biomarkers. As with many disease conditions finding specific and sensitive markers that are associated with only one type of the disease can be difficult. In addition to this, the level of the biomarkers in biological fluids can vary depending on different disease conditions and stages. A number of molecular markers are therefore usually evaluated for cancer diagnosis and these can include proteins, peptides, over/under expression of gene markers and gene mutations. This review provides an overview of the biosensor technology available today, areas which are currently being developed and researched for cancer markers diagnosis—and a consideration of future prospects for the technology.



**Contents**



## 1. Introduction

The early diagnosis of cancer is crucial for patient survival and successful prognosis of the disease—and for this reason sensitive and specific methods are required for early cancer diagnosis. Cancer continues to be the most feared global disease, with prostate, lung, breast, and colon cancer toping the list of cause of mortality in the United State and Canada in 2006 [1,2]. In the UK in 2006 there were 154,162 deaths from cancer (Cancer Research UK [3]). Collectively cancer has also been identified as the second largest cause of death in most developing countries. The analysis of biomarkers in blood, urine and other body fluids is one of the methods applied in the detection of the disease. Multi-marker profiles (presence and concentration level) can be essential for the diagnosis of early disease onset. These methods should provide information to assist clinicians in making successful treatment decisions and increasing patient survival rate. Existing methods of screening for cancer rely heavily on traditional methods which are based on cell morphology

* Tel.: +44 7500 766487.
*E-mail address:* i.tothill@cranfield.ac.uk.

1084-9521/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.semcdb.2009.01.015

using staining and microscopy. These are invasive techniques based on taking a biopsy and then examining the tissue using cell fixation and morphology approaches to identify and detect cancer cells. These tests, however, are not individually conclusive as tissue removal can miss cancer cells at the earlier onset of the disease and further research is needed to develop tests to identify the malignancy without the need for invasive examinations. More recent techniques use immunoassays (e.g. ELISA) to detect cancer biomarkers and these are carried out in hospitals laboratories. However, in most cases only one biomarker is used as an indicator of the disease. Immunoassays are very sensitive and selective, but can be time consuming and expensive. In addition to this some ELISA tests are not sufficiently sensitive for the detection of low level marker concentrations which exist in the early stages of the disease. The use of molecular tools (genomic and proteomics) to create molecular signatures of the disease have been employed recently. These techniques are producing data which are also useful in understanding the disease, but are complex and require data management as well as linking to patient information. A more sensitive and rapid technology platform is therefore needed to fulfil the rapid diagnosis requirements in cancer detection. Rapid diagnosis will help in providing better health care and reducing the waiting time for results dissemination—which can be highly stressful to the patients.

Cancer can be caused by a range of factors and these include genetic or environmental factors such as the exposure to carcinogenic chemicals, radiation or microbiological causes including: bacterial (e.g. stomach cancer) or viral infections (e.g. cervical cancer). As the causes of cancer are so diverse, clinical testing is also very complex. There are more than 200 distinct diseases associated with cancer, affecting different part of the body. In cancer, the disruption of the normal cell signalling pathways leads to the inactivation of the tumour suppressor genes and the activation of oncogenes. These multi-factorial changes (genetic and epigenetic) can cause the onset of the disease and the formation of cancer cells which will exhibit higher growth capability than normal mammalian cells. However, in term of diagnosis, no single gene is universally altered during this process, and the patterns of change differ in tumours from different locations (organ), as well within tumours from the same location [4]. All these changes which take place can be so variable and overlapping that it is difficult to select a specific change or marker for the diagnosis of specific cancers. Therefore, a range of biomarkers can potentially be analysed for disease diagnosis.

New and emerging molecular methods are being used to study cancer and these are resulting in a better understanding of the disease as well as the discovery of potential new genomic and proteomic biomarkers. Multi-analyte analysis based on a lab-on-a-chip point-of-care devices (POC) are needed to overcome the challenges in cancer diagnosis [5]. To date research in this area is expanding rapidly and a vast number of new technologies are being developed in the diagnostics arena. A range of biosensor platforms are reported in the literature for cancer disease diagnosis. However, moving the technology to commercial products may require more time and investment. The discovery of new molecular markers which are very specific and sensitive can help in making diagnosis non-invasive. In this review different markers and biosensor platforms available for cancer diagnosis, prognosis and disease recurrence monitoring in the form of point-of-care, are covered and discussed.

## 2. Cancer markers

Tumour associated antigens have been used as biomarkers for cancer diagnosis. These comprise cellular molecules that can be detected in tumour cells, blood, urine, or other body fluids which are over-expressed due to cancer onset and growth. To date there are a range of biomarkers which have been identified with different types of cancers. These include DNA modifications, RNA, proteins (enzymes and glycoproteins), hormones and related molecules, molecules of the immune system, oncogenes and other modified molecules. New advances in proteomics and genomics research are resulting in the elucidation of new biomarkers. Combining this with toxicology studies and high throughput strategies, multiple antigens can be identified and used as biomarkers for diagnosis. Protein micro-arrays, serological analysis of recombinant cDNA expression libraries (SEREX) as well as serological proteome analysis (SERPA) technologies have been some of the chosen strategies used to identify tumour associated antigens [2]. A range of cancer markers have been identified using SEREX such as the breast cancer antigens NY-BR-1 and ING-1 and cancer testis antigens, CAGE-1 and ESO-1 [6–8]. A number of genes have been identified as being associated with specific cancers and can be classified as accurate predications of risk. Hereditary cancers include those associated with colorectal cancer with mutation in the HNPCC or FAP gene—and breast–ovarian cancer with mutations in BRCA 1/2 genes. Biomarkers which have been marked as being reliable for diagnosis and prognosis of the disease are listed in several reference cancer databases (e.g. National Centre for Biotechnology Information (NCBI), Cancer Research UK [3]) and literature [4,9,10]. Table 1 shows a collated list of markers associated with different cancers. Markers can be used for early diagnosis, disease monitoring and prognosis as well as predictive markers. Many markers are still going through evaluation to improve their specificity and sensitivity in the context of their clinical use [11]. Guidelines have also been developed for the best way to report tumour markers discovery studies, so that these studies can be transparent and easily understood in the context of their conclusions [12].

**Table 1**
Known biomarker associated with cancer diagnosis and prognosis.

| Cancer type disease | Biomarker |
|---|---|
| Prostate | PSA, PAP |
| Breast | CA15-3, CA125, CA27.29, CEABRCA1, BRCA2, MUC-1, CEA, NY-BR-1, ING-1 |
| Leukaemia | Chromosomal abnormalities |
| Testicular | α-Fetoprotein (AFP), β-human chorionic gonadatropin, CAGE-1, ESO-1 |
| Ovarian | CA125, AFP, hCG, p53, CEA |
| Any solid tumour | Circulating tumour cells in biological fluids, expression of targeted growth factor receptors |
| Colon and pancreatic | CEA, CA19-9, CA24-2, p53 |
| Lung | NY-ESO-1, CEA, CA19-9, SCC, CYFRA21-1, NSE |
| Melanoma | Tyrosinase, NY-ESO-1 |
| Liver | AFP, CEA |
| Gastric carcinoma | CA72-4, CEA, CA19-9 |
| Esophagus carcinoma | SCC |
| Trophoblastic | SCC, hCG |
| Bladder | BAT, FDP, NMP22, HA-Hase, BLCA-4, CYFRA 21-1 |

Biomarkers can either be present inside the cancer cells or be extracellular. If the markers are inside the tumour cells, then these need to be collected and enriched (if the concentration is very low) with the cells needing to be lysed to release the biomarkers before analysis. Enrichment methods to select tumour cells have been reported and these include immunomagnetic separation using magnetic beads [13–15] and centrifugation using a density gradient [16]. The thresholds of some basic tumour markers in human serum are listed in Table 2 [9,10].

Over-expressed proteins as a result of cancer cells growth have been employed as biomarkers for cancer diagnosis. As an example, prostate specific antigen (PSA), which is responsible for the liquefaction of seminal fluid and is also present in the serum of male patients, is used as a biomarker [17]. The level of PSA can be markedly raised in the serum due to prostate cancer. Therefore, this protein has been used as a marker for prostate cancer for both diag-

**Table 2**
The thresholds of basic tumour markers in human serum [9,10].

| Tumour markers | Thresholds | Tumour markers | Thresholds |
|---|---|---|---|
| NSE | 12.5 mg $l^{-1}$ | CEA | 3 μg $l^{-1}$ |
| PSA | 4 μg $l^{-1}$ | CA125 | 35 kU $l^{-1}$ |
| GST | 3.2 U $l^{-1}$ | CA153 | 25 kU $l^{-1}$ |
| ALP | ~0 | CA27-29 | 36.4 kU $l^{-1}$ |
| CT | 100 ng $^{-1}$ | CA549 | 11 kU $l^{-1}$ |
| SCCA | 1.5 μg $l^{-1}$ | CA19-9 | 37 kU $l^{-1}$ |
| Ferritin | 250 μg $l^{-1}$ | CA50 | 14–20 kU $l^{-1}$ |
| hCG | 5.0 IU $l^{-1}$ | CA242 | 20 kU $l^{-1}$ |
| AFP | 10 μg $l^{-1}$ | CA72-4 | 6 kU $l^{-1}$ |

nosis and monitoring. However, although PSA is a highly sensitive marker its specificity is low. Therefore, a multi-marker diagnosis may aid in the increase of the specificity of point-of-care tests used for prostate cancer detection. For breast cancer for example, CA 15-3, estrogen receptor, progesterone receptor and her2-neu are all proteins associated with this disease—while for ovarian cancer, CA125 is a protein marker. New fields of cancer biomarker discovery also include monitoring of volatiles in blood and urine as indicators of the disease. This research area is in its infancy and therefore volatile biomarkers which are associated with cancer are still limited. As an example, studies on breast cancer have seen the utilisation of alkanes, methylated alkanes and conjugated dienes as markers of oxidative stress—and have been shown to exhibit some diagnostic specificity [18].

Molecular tools (genomic and proteomic) such as PCR, Southern blotting, DNA sequencing, etc. are being used to investigating DNA methylation and biomarkers discovery. The use of reverse transcriptase polymerase chain reaction (RT-PCR) assays for cancer genes expression analysis is also increasing. This test is used for predicting increase risk of disease in patients [19]. However, the above procedures are lab based, complex, slow and require experienced personal to conduct the analysis. They are also reported to not be sufficiently precise for clinical applications [20]. Therefore, the development of protein based biomarkers for disease diagnosis rather than genetic markers has significantly increased in recent years. The use of biosensors for protein biomarkers analysis has therefore been further highlighted as an attractive and cost effective technique for the development of point-of-care devises.

## 3. Biosensors as diagnostics tools

A biosensor is defined as a bioanalytical device incorporating a molecular recognition entity associated with or integrated with a physicochemical transducer (Fig. 1). Biosensors can be classified as point-of-care devices (POC) which can bring the capability of analysing clinical samples in the home or at the doctor's surgery. In order to develop appropriate biosensor technologies, specific markers need to be identified to ensure specificity of the devices. Biosensors provide advanced platforms for biomarker analysis with the advantages of being easy to use, inexpensive, rapid and robust as well as offering multi-analyte testing capability. For cancer diagnosis multi-array sensors would be beneficial for multi-marker diagnosis (multiplexing capabilities). Sample preparation is usually incorporated as part of the sensor system such as glucose sensors which contain microfluidics and membrane separation systems for blood samples handling.

### 3.1. Molecular recognition element

In order to recognise specifically the cancer biomarker, the optimal recognition materials should be implemented as the receptor molecule in the biosensor design. This is especially important for medical diagnosis since the sensitivity and specificity of the sensing molecules will play an important part in the success of the sensor device. A range of molecular recognition entities have been used for biomarker detection. The most widely used is the antibody molecule, which provides the specificity and sensitivity required for low levels of molecular detection. More recently, synthetic (artificial) molecular recognition elements have been fabricated as affinity materials and used for analyte detection and analysis. These type of materials can include nanomaterials and membrane structures and can comprise molecular imprinted polymers (MIPs), aptamers, phage display peptides, binding proteins and synthetic peptides as well as metal oxides materials [21–24].

Antibodies (monoclonal and polyclonal) have been applied in cancer treatment as therapeutics targeting tumours inside the body and are also used in diagnostics tests targeting cancer cells and biomarkers [25]. Polyclonal antibodies can be raised against any biomarker or cells and with the introduction of high throughput techniques, applying these molecules in sensors has been successful [26]. The use of monoclonal antibodies however, results

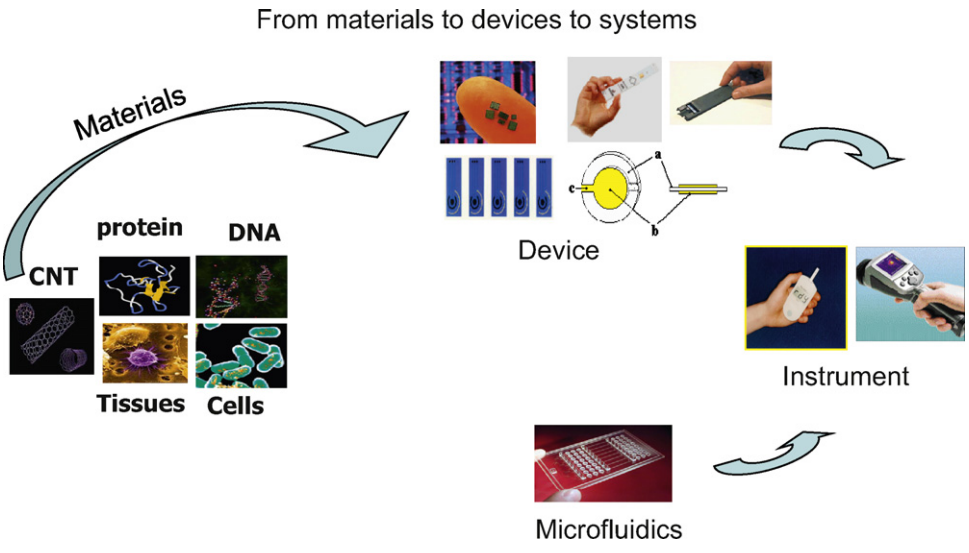


**Fig. 1.** Construction of a biosensor.

in more specific tests. The drawbacks include that monoclonal antibodies are more difficult to maintain and can be more expensive than polyclonal antibodies. A range of antibodies are now commercially available for specific cancer markers, and with the emergence of new markers, new antibodies can be raised specifically for them. Many ELISA tests, dipsticks and assays have been developed for cancer diagnosis using antibodies. Antibody fragments and molecular-engineered antibodies have been developed for biosensor applications. The use of direct and combinatorial mutagenesis has been applied to enhance the affinity and selectivity of recombinant antibodies. However, a lack of stability of the antibody molecule makes this approach unsuitable for long term device storage and hinders wider application and commercialisation. Replacing natural biomolecules with artificial receptors or biomimics has therefore become an attractive area of research in recent years [27]. The advantages of using these molecules are that they are robust, more stable and inexpensive to produce since they can be made using wet chemistry approaches and can be modified easily to aid immobilisation on the sensor surface as well as adding labels as the maker for detection. They also obviate the use of animals in the production of affinity receptors as is the case in polyclonal antibodies.

The use of phage display technology to screen libraries for specific receptor molecules is being rapidly applied in the diagnostics arena. This is to identify specific peptide sequences with affinity towards target analytes [28–32]. Peptide ligands have been identified from screening phage display libraries that target specific cancer biomarkers, such as HER2/neureceptor, ErbB-2, and ICAM-1 [33,34]. Combinatorial libraries with peptides synthesised on beads are mainly used for drug discovery applications but more recently they have also been applied for receptor ligands selection by screening them against specific analyte targets [35,36]. Peptides that recognise the $\alpha_6\beta_1$ integrin expressed on the prostate cancer line DU145 have also been identified [37]. Peptides can be chemically synthesised in large quantities once the optimal peptide with the required affinity has been identified. However, peptides have relatively lower affinity ($K_d \sim 10^{-6}$ to $10^{-7}$ M) when compared to antibodies and therefore designing the assay is important for developing successful sensing methods when incorporating these in sensors. Research in the development of higher affinity peptide as ligands also needs further investment.

Aptamers (derived from the latin *aptus*, meaning 'to fit') are synthetic nucleic acids ligands (DNA or RNA) which can be selected from combinatorial libraries of synthetic nucleic acids, to have specific affinity for a target molecule such as cancer biomarkers. Using synthetic evolution of ligands (SELEX), process aptamers can be selected from random pools and produced [38,39]. These can be selected against drugs, proteins and other analytes and used as receptors in sensor devices [40]. Aptamers are attracting interest in the area of diagnosis and are being developed to be implemented in future devices. Aptamers have been selected that claimed to have high affinity ($K_d \sim 10^{-8}$ to $10^{-9}$ M) for target molecules. However, their specificity in most cases is lower than antibodies. Detection of aptamer–protein interactions using label-free electrochemical detection systems based on impedance spectroscopic transduction have been reported [41].

The use of organic polymers to specifically imprint the target molecule has been carried out and the production of molecular imprinted polymers as synthetic receptor has been realised [42,43]. Molecular imprinting polymerisation has been the focus of intense research interest in the past 10 years and to date is being considered in a wide range of application areas, e.g. in the preparation of selective separation materials and as sensing layers in sensor devices [44,45]. MIPs are usually produced by forming a highly cross-linked organic polymer around the molecule of interest (analyte target/template). A recognition of proteins such as cancer biomarkers using molecular imprinting polymers is relatively a new area. A recent example is the work reported by Jégourel et al. [46], who developed a molecular imprinted polymer for extracting pyrimidine nucleoside cancer markers in urine. Attempts at printing of amino acids derivatives [47] small peptides [48] and proteins [49] have all been undertaken with limited success.

The construction of peptide receptors based on the molecular imprinting approach has recently been carried out [50,51]. In this approach amino acids are used as the functional monomers for the polymerisation process around the template which is the molecular analyte target. The use of molecular modelling has been applied for the selection of the highest affinity monomers to be used in the construction of MIPs. The construction of virtual libraries of functional monomers against target molecules followed by the selection of the highest binding score monomers as the building blocks has been undertaken within many studies [52–55]. The software facilitates calculations using different dielectric constants to reflect the polarity of the environment (solvents) in which the polymers have to be prepared or used. Molecular imprinted polymers are conceptually attractive due to their ease of preparation, high thermal and chemical stability and long shelf-life in conditions of ambient temperature and humidity. However, the major disadvantage is that MIPs require a template (pure form of the analyte) to produce the synthetic polymer which can in some cases represent a significant cost. Also their application as sensing layers in sensor devices has been limited due to their low specificity and sensitivity when compared to the antibody molecule.

### 3.2. Transducers

A transducer is the device that converts recognition signal events into electrical (often digital) signals—and can be electrochemical (amperometry, potentiometry, conductimetry/impedimetry), optical (colorimetric, fluorescence, luminescence, interferometry), calorimetric (thermistor), mass change (piezoelectric/acoustic wave) or magnetic in nature [56]. Electrochemical transducers are the most widely used in sensor technology, however recently, optical and QCM type of sensors are becoming more popular and their use is expanding in practical applications. The main reason for this is that most electrochemical systems still require the use of a reporter molecule such as enzyme labels where a substrate mixture needs to be added for the assay results to be monitored. In an optical system the binding event between the receptor used on the sensor surface and the biomarker can be detected directly. In a piezoelectric/acoustic system, the change in mass due to the binding between the biomarker and the receptor on the sensor can also be monitored directly. A range of sensors have been developed for cancer markers diagnosis and these have been reviewed by several authors [9,10,4,57,58].

#### 3.2.1. Electrochemical transducer

Electrochemical biosensors are used in point-of-care devices since they are portable, simple, easy to use, cost effective and in most cases disposable. The electrochemical instruments used with the biosensors have been miniaturised to small pocket size devices which make them applicable for home use or the doctor's surgery. The glucose biosensor is the most widely used example of an electrochemical biosensor which is based on a screen-printed amperometric disposable electrode. This type of biosensor has been used widely throughout the world for glucose testing in the home bringing diagnosis to on site analysis. Another development in this area is the hand held i.STAT clinical analyser which combines several electrochemical biosensors on a single chip and is used for multiple electrolytes and metabolites in clinical samples. Electrochemical affinity sensors based on antibodies offer great selectivity and sensitivity for early cancer diagnosis. A range of electrochemical

transducers are used and these include amperometric, potentiometric and impedimetric/conductivity devices.

Amperometric and potentiometric transducers have been the most commonly used, but much attention in recent years has been devoted to impedance based transducers since they are classified as label-free detection sensors. Recent reviews in the use of electrochemical biosensors for cancer diagnosis have been reported by Lin and Ju [58] and Wang [59]. For cancer biomarkers analysis, bioaffinity based electrochemical biosensors are usually applied to detect gene mutations of biomarkers and protein biomarkers. However, much of the technology is still at the research stage. Electrochemical devices have been developed based on DNA hybridisation and used for cancer gene mutation detection. In this type of device a single stranded DNA sequence is immobilised on the electrode surface and when DNA hybridisation takes place, detection is conducted using a range of methodologies. Techniques which have been used include: the use of guanine oxidation (label-free); the use of enzyme or redox labels to generate the signal or changes in the electrochemical signal due to changes in conductivity or capacitance due to the formation of DNA duplexes on the electrode surface. Wang and Kawde [60] employed chronopotentiometric transduction for label-free guanine based electrochemical detection of mutations related to breast cancer genes BRCA1 and BRCA2. Tansil et al. [61] also reported the detection of cancer marker genes from breast tissues using the catalytic oxidation of the guanine nucleobase.

ELISA based assays conducted on the electrode surface are the most frequently used techniques for cancer protein markers analysis. In this method the antibody (or antigen) is labelled with an enzyme such as horseradish peroxidase (HRP), or alkaline phosphatase (AP) and these will then catalyze an added substrate to produce an electroactive species which can then be detected on an electrochemical transducer. Electrochemical detection of protein markers is more widely used for cancer analysis. Changes in the electrochemical properties (conductivity or capacitance) on the sensor surface due to the antibody antigen interaction can also be used as the detection principle. The use of Faradic impedance spectroscopy has been reported for the analysis of CEA biomarkers using gold nanoparticle modified glassy carbon electrodes [62]. Table 3 lists examples of electrochemical biosensors which have been developed for cancer biomarkers analysis. Patterns of protein profile can be detected on a single chip with multi-array working electrode, where different antibodies are immobilised to detect a specific marker. These types of transducers can be easily fabricated using screen-printing technology or via the use of semiconductor chips with macro/micro-array patterns. In order to develop practical devices for commercial developments, problems of binding between heterogeneous antigens and antibodies used in the sensor assays—and also the high background signals need to be eliminated and controlled.

### 3.2.2. Optical transducers

Optical transducers used in biosensors include fluorescence, interferometry and spectroscopy of optical waveguides and surface plasmons resonance (SPR). Many commercially available platforms use fluorescence labels as the detection system. However, the instruments used for signal readout are usually expensive and are

**Table 3**
Examples of biosensors for cancer biomarkers analysis.

| Cancer marker detected | Biosensor principle | Assay principle | Limit of detection | Reference |
|---|---|---|---|---|
| AFP | Electrochemical | Protein array with 36 platinum electrodes. | – | [95] |
| | | Prussian blue with screen-printed amperometric sensor. | (range 5–500 ng ml$^{-1}$) | [96] |
| AFP and CEA | Electrochemical | Dual-electrode with amperometric detection. | 1 ng ml$^{-1}$ | [71] |
| CA15-3 | Electrochemical | Antibody functionalised sol–gel film with potentiometric detection. | 5 U ml$^{-1}$ | [97] |
| CA125 | Electrochemical | Capillary electrophoretic. | – | [98] |
| | | Titania sol–gel on glassy carbon electrode with direct electrochemical detection of HRP. | 1.29 U ml$^{-1}$ (range 2–14 U ml$^{-1}$) | [99] |
| CA19-9 | Electrochemical | Titania sol–gel on carbon electrode as an amperometric immunosensor. | – | [100] |
| CEA | Electrochemical | Faradic impedance spectroscopy using gold nanoparticle modified glassy carbon electrode. | – | [62] |
| | | Immobilised thionine as a mediator between the electrode and HRP-labelled antibody. | (range 0.6–17 ng ml$^{-1}$, 17–200 ng ml$^{-1}$) | [101] |
| | | Direct electrochemical detection of HRP in an immunosensor. | 0.4 ng ml$^{-1}$ (range 0.5–3.0 ng ml$^{-1}$, 3.0–120 ng ml$^{-1}$) | [102] |
| | Optical | Chemiluminescence immunosensor. | – | [103] |
| Ferritin | Mass sensitive | Antibody immobilised on gold chip of a quartz crystal microbalance. | (range 0.1–100 ng ml$^{-1}$) | [67] |
| | Optical | SPR based immunosensor. | – | [104] |
| hCG | Mass sensitive | A multi-channel piezoelectric quartz micro-array immunosensor. | – | [68] |
| | Optical | Fluorescence immunosensor. | (range 25–1500 U ml$^{-1}$) | [105] |
| PSA | Electrochemical | Gold coated microporous membrane. | – | [106] |
| | | Amperometric disposable electrode. | 0.25 ng l$^{-1}$ | [107] |
| | | Capacitive immunosensor using lateral flow and impedance detection. | – | [108] |
| | Mass sensitive | Antibody immobilised on gold chip of a quartz crystal microbalance. | – | [66] |
| | | Microcantilever immunosensor. | – | [109] |
| | | Microcantilever immunosensor. | (range 0.2–60 μg ml$^{-1}$) | [80] |
| | Optical | SPR with colloidal gold nanoparticles. | 0.15 ng ml$^{-1}$ | [110] |

more suitable for laboratory settings. As an example the Affymetrix gene chip (Affymetrix Inc., Santa Clara, USA) can be used for screening cancer for cancer gene identification. Other biosensor platforms such as grating couplers, resonant mirrors and surface plasmon based systems have also been used for cancer biomarkers diagnosis. These are classified as label-free and real-time affinity reaction detection systems. SPR systems such as the BiaCore™ biosensor chip (GE Health Care, USA) are amongst the most widely used optical transducers used as a biosensor platform. Their use for cancer biomarkers detection has been reported in several applications. Other companies have also produced SPR sensors including Texas Instruments, Nippon Laser and Electronics Laboratories. Different SPR based biosensors have been developed for cancer markers detection based on the above optical systems, some of which are reported in Table 3. However, these sensors are usually more suitable for laboratory based testing rather than point-of-care devices for on site analysis.

*3.2.3. Mass sensitive transducer*

This type of transducer is also classified as being a label-free technology. Piezoelectric sensors comprise a quartz crystal coated with a gold electrode and are used as microbalances (QCM) sensitive for a changes in the mass on the sensor surface [63]. QCM devices have been used for a wide range of applications in the medical field [64,65]. Piezoelectric immunosensors are usually designed to detect cancer markers where the specific antibody is immobilised on the sensor chip. More advanced devices are now available on the market based on this technology. One example is the QCMA 1 Sensor instrument (Sierra Sensors GmbH, Hamburg, Germany) which is fully automated. This is being used at Cranfield University (England, UK) to develop an affinity sensor for PSA analysis. The assay is based on the use of a gold chip with antibody immobilised on the surface of the sensor. Good sensitivity has been reported using this system [66]. Piezoelectric immunosensors for human ferritin [67] and hCG [68] have also been proposed.

Recently, microcantilever based sensors have also been applied for cancer biomarkers detection. Affinity interactions between the antibody on the surface of the cantilever and the biomarker are detected through the amount of bending of the sensor due to mass changes which are detected as changes in the resonant frequency [69]. Table 3 also lists examples of mass sensitive transducers based sensors.

*3.2.4. Simultaneous multi-marker detection*

Multi-cancer markers based detection systems have also been reported in the literature. Many are based on the use of multi-ELISA assays on the same chip to detect several analytes at the same time by using the immunosensor principle. Wu et al. [9,10] reported on the development of a disposable electrochemical immunosensor for simultaneous determination of CA19-9 and CA125—with limits of detection of 0.2 and 0.4 U $ml^{-1}$ respectively. A flow-through multi-analyte sensor for CA125 and CEA was developed by Fu et al. [70]. Other examples of multi-marker sensors include those reported by Wilson [71], Wilson and Nie [72].

## 4. Nanomaterials application in biosensing

The use of nanomaterials and structures such as semiconductors and conducting polymer nanowires for biosensor applications is expanding rapidly and many comprehensive review articles are now published such by Willner et al. [73] and Katz et al. [74]. Nanotechnology application in biosensors development can range from the transducer device, the recognition ligand, the label and the nanorunning systems. Their increased use is due to the excellent advantages offered by these materials via miniaturisation of the devices, signal enhancements and amplification of the signal by nanoparticle labels which increase sensitivity and also allow the fabrication of multiplex sensor systems such as high density protein arrays [75,76]. The high surface to volume ratio offered by nanomaterials makes these devices very sensitive and can allow single molecule detection which is very attractive in cancer diagnosis. The use of nanowire transducers can offer greater sensitivity in affinity sensors [77,78]. Microcantilever devices which bend due to changes in surface mass as a result of affinity reactions are also being developed and applied for cancer biomarkers detection [79,80].

The use of luminescent nanocrystals (Quantum dots) as molecular labels to replace fluorophores has created new applications for nanomaterials in cellular labelling and visualisation [81–84]. These nanocrystals can be attached to molecules for intracellular tracking or as labels for antibodies and other molecules to detect cancer cells or biomarkers. Quantum dots show distinct advantages over other markers due to their spectroscopic properties and narrow emission peaks and for this reason their use in multiplexed analysis is increasing. Their high emission quantum yield results in improved signal/noise ratios and therefore a decrease in false readings (negative and positive).

The use of striping voltammetry for detecting metal nanoparticals has been applied where these metals have been used as marker tags. Gold and silver nanoparticals can be used in these methods including different inorganic nanocrystals (e.g. ZnS, PbS, and CdS) for detecting biomarkers. Several products are available on the market such as Oxanica (UK) Quantum dots and MultiPlxBeads™ from Crystalplex Corp., USA. Oxanica also market the NANOPLEX™ product which is based on a lateral flow device and uses nanoparticals for biomarker detection. Nanoparticals can also be exploited in conductivity based sensors where they can induce a change in the signal upon the attachment of the nanoparticle–antibody conjugate, tagged with the captured antigen on the sensor surface. Choi et al. [85] reported the use of gold nanoparticles to detect prostate specific antigen using SPR. This sensor system showed a detection limit of 300 fM of the cancer marker.

The development of micro/nanosensor devices for cancer marker diagnosis is increasing due to their extremely attractive characteristics for this application. Their novel electron transport properties make them highly sensitive for low level detection [86,87]. The multiplex analysis capability is also very attractive for multi-biomarker analysis. The use of antibody functionalised silicon chips as sensor arrays, conducting polymer nanowires and carbon-nanotubes have been reported recently for cancer biomarkers diagnosis [88–90].

## 5. Microfluidics

In order to develop a hand held biosensor device that can be used outside the laboratory, a microfluidic system needs to be incorporated as part of the sensor device. Integrated microfluidics will enable samples and fluids to move in point-of-care devices (lab-on-a-chip) so that the reaction takes place efficiently on the sensor surface [91,92]. Advances in the development of microfluidics is increasing and different moulding and fabrication technologies have been applied, such as photolithography and using quartz, silica, glass or composite materials and chips. These approaches will then produce a fully integrated lab-on-a-chip system that allows multi-analyte analysis. Systems for cell separation from blood samples and other fluids have been reported in the literature [93,94]. However, most of the multi-array sensor chips for on site sensing are still in development and in the research stage.

## 6. Conclusions

It is crucial to diagnose cancer early for the successful treatment and recovery of patients suffering from the disease. There is there-

fore a need for simple and sensitive diagnostics methods that can detect multiple cancer biomarkers that exist at low concentrations in biological fluids. Biosensors can fulfil these requirements [111]. However, biosensor devices need to be further developed to face these new challenges to allow, for example, multiplex analysis of several biomarkers where arrays of sensors need to be developed on the same chip. Future innovation in biosensor technology to include biomarkers patterns software and microfluidics can make these devices of high potential for this application area. The concept of using nanomaterials in the development of sensors for biomarkers diagnosis will make these devices highly sensitive and more applicable for point-of-care early diagnosis. Early diagnosis will aid in the increase in the survival rate and successful development of biosensors for cancer diagnosis will require appropriate funding to move the technology from research through to the realisation of commercial products.

## References


[1] Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, et al. Cancer statistics. CA Cancer J Clin 2006;56:106–30.
[2] Gunawardana CG, Diamandis EP. High throughput proteomic strategies for identifying tumour-associated antigens. Cancer Lett 2007;249:110–9.
[3] Cancer Research UK. November 8, 2008, http://info.cancerresearchuk.org/cancerstats/mortality/.
[4] Soper SA, Brown K, Ellington A, Frazier B, Garcia-Manero G, Gau GV, et al. Point-of-care biosensor systems for cancer diagnostics/prognostics. Biosens Bioelectron 2006;21:1932–42.
[5] Ahn CH, Choi JW, Beaucage G, Nevin JH, Lee JB, Puntambekar A, et al. Disposable smart lab on a chip for point-of-care clinical diagnostics. Proc IEEE 2004;92:154–73.
[6] Chen YT, Scanlan MJ, Sahin U, Tureci AO, Gure ST, Williamson B, et al. A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. Proc Natl Acad Sci USA 1997;94:1914–8.
[7] Jager D, Stockert E, Gure AO, Scanlan MJ, Karbach J, Jager E, et al. Identification of a tissue-specific putative transcription factor in breast tissue by serological screening of a breast cancer library. Cancer Res 2001;61:2055–61.
[8] Parker S, Lim Y, Lee D, Cho B, Bang YJ, Sung S, et al. Identification and characterisation of a novel cancer/testis antigen gene CAGE-1. Biochim Biophys Acta 2003;1625:173–82.
[9] Wu J, Fu Z, Yan F, Ju H. Biomedical and clinical applications of immunoassays and immunosensors for tumor markers. Trends Anal Chem 2007;26:679–88.
[10] Wu J, Zhang Z, Fu Z, Ju H. A disposable two-throughput electrochemical immunosensor chip for simultaneous multianalyte determination of tumor markers. Biosens Bioelectron 2007;23:114–20.
[11] Sanchez-Carbayo M. Recent advances in bladder caner diagnostics. Clin Biochem 2004;37:562–71.
[12] McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM. Reporting recommendations for tumour MARKer prognostic studies (REMARK). Eur J Cancer 2005;41:1690–6.
[13] Hu XC, Wang Y, Shi DR, Loo TY, Chow LW. Immunomagnetic tumor cell enrichment is promising in detecting circulating breast cancer cells. Oncology 2003;64:160–5.
[14] Lara O, Tong X, Zborowski M, Chalmers JJ. Enrichment of rare cancer cells through depletion of normal cells using density and flow-through, immunomagnetic cell separation. Exp Hematol 2004;32:891–904.
[15] Zieglschmid V, Hollmann C, Gutierrez B, Albert W, Strothoff D, Gross E, et al. Combination of immunomagnetic enrichment with multiplex RT-PCR analysis for the detection of disseminated tumor cells. Anticancer Res 2005;25:1803–10.
[16] Choesmel V, Pierga JY, Nos C, Vincent-Salomon A, Sigal-Zafrani B, Thiery JP, et al. Enrichment methods to detect bone marrow micrometastases in breast carcinoma patients: clinical relevance. Breast Cancer Res 2004;6:R556–70.
[17] Eble JN, Sauter G, Epstein JI, Sesterhenn IA. World Health Organization classification of tumours. pathology and genetics of tumours of the urinary system and male genital organs. Lyon: IARC Press; 2004.
[18] Phillips M, Cataneo R, Ditkoff B, Fisher P, Greenberg J, Gunawardena R, et al. Volatile markers of breast cancer in the breath. Breast J 2003;9(3):184–91.
[19] Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med 2004;351:2817–26.
[20] Ross JS, Fletcher JA, Bloom KJ, Linette GP, Stec J, Symmans WF, et al. Targeted therapy in breast cancer. Mol Cell Proteomics 2004;3:379–98.
[21] Tothill IE. Biosensors developments and potential applications in the agricultural diagnosis sector. Comput Electron Agric 2001;30:205–18.
[22] Tothill IE, Piletsky S, Magan N, Turner APF. New biosensors. In: Kress-Rogers E, Brimelow CJB, editors. Instrumentation and sensors for the food industry. 2nd ed. Woodhead Publishing Limited and CRC Press LLC; 2001. p. 760–76.
[23] Shaikh KA, Ryu KS, Goluch ED, Nam JM, Liu J, Thaxton CS, et al. A modular microfluidic architecture for integrated biochemical analysis. Proc Natl Acad Sci USA 2005;102:9745–50.
[24] Collett JR, Cho EJ, Lee JF, Levy M, Hood AJ, Wan C, et al. Functional RNA microarrays for high-throughput screening of antiprotein aptamers. Anal Biochem 2005;338:113–23.
[25] Green MC, Murray JL, Hortobagyi GN. Monoclonal antibody therapy for solid tumors. Cancer Treat Rev 2000;26:269–86.
[26] Chambers RS, Johnston SA. High-level generation of polyclonal antibodies by genetic immunization. Nat Biotechnol 2003;21:1088–92.
[27] Tothill IE. On-line immunochemical assays for contaminants analysis. In: Tothill IE, editor. Rapid and on-line instrumentation for food quality assurance. Woodhead Publishing Limited; 2003. ISBN: 1-85573-674-8.
[28] Oyama T, Sykes KF, Samli KN, Minna JD, Johnston SA, Brown KC. Isolation of lung tumor specific peptides from a random peptide library: generation of diagnostic and cell-targeting reagents. Cancer Lett 2003;202:219–30.
[29] McGuire MJ, Samli KN, Johnston SA, Brown KC. In vitro selection of a peptide with high selectivity for cardiomyocytes in vivo. J Mol Biol 2004;342:171–82.
[30] McGuire MJ, Sykes KF, Samli KN, Timares L, Barry MA, Stemke-Hale K, et al. A library-selected, Langerhans cell-targeting peptide enhances an immune response. DNA Cell Biol 2004;23:742–52.
[31] Chang CY, Abdo J, Hartney T, McDonnell DP. Development of peptide antagonists for the androgen receptor using combinatorial peptide phage display. Mol Endocrinol 2005;19:2478–90.
[32] Shukla GS, Krag DN. Selection of tumor-targeting agents on freshly excised human breast tumors using a phage display library. Oncol Rep 2005;13:757–64.
[33] Belizaire AK, Tchistiakova L, St-Pierre Y, Alakhov V. Identification of a murine ICAM-1-specific peptide by subtractive phage library selection on cells. Biochem Biophys Res Commun 2003;309:625–30.
[34] Karasseva NG, Glinsky VV, Chen NX, Komatireddy R, Quinn TP. Identification and characterization of peptides that bind human ErbB-2 selected from a bacteriophage display library. J Protein Chem 2002;21:287–96.
[35] Brown KC. New approaches for cell-specific targeting: identification of cell-selective peptides from combinatorial libraries. Curr Opin Chem Biol 2003;4:16–21.
[36] Chen B, Bestetti G, Turner APF. The synthesis and screening of a combinatorial library for affinity ligands for glycosylated haemoglobin. Biosens Bioelectron 1998;13:779–85.
[37] DeRoock IB, Pennington ME, Sroka TC, Lam RS, Bowden GT, Bair EL, et al. Synthetic peptides inhibit adhesion of human tumor cells to extracellular matrix proteins. Cancer Res 2001;61:3308–13.
[38] Ulrich H, Alves MJM, Colli W. RNA and DNA aptamers as potential tools to prevent cell adhesion in disease. Braz J Med Biol Res 2001;34:295–300.
[39] Cerchia L, Hamm J, Libri D, Tavitian B, de Franciscis V. Nucleic acid aptamers in cancer medicine. FEBS Lett 2002;528:12–6.
[40] O'Sullivan CK. Aptasensors—the future of biosensors? Anal Bioanal Chem 2002;372:44–8.
[41] Rodriguez M, Kawde N, Wang J. Aptamer biosensor for label-free impedance spectroscopy detection of proteins based on recognition-induced switching of surface change. Chem Commun 2005;34:4267–9.
[42] Mosbach K. Molecular imprinting. Trends Biochem Sci 1994;19:9–14.
[43] Mosbach K, Ramstrom O. The emerging technique of molecular imprinting and its future impact on biotechnology. Biotechnology 1996;14:165–70.
[44] Piletsky S, Piletska EV, Sergeyeva TA, Nicholls I, Weston D, Turner A. Synthesis of biologically active molecules by imprinting polymerization. Biopolym Cell 2006;22:63–8.
[45] Piletsky S, Turner NW, Laitenberger P. Molecularly imprinted polymers in clinical diagnostics—future potential and existing problems. Med Eng Phys 2006;28:971–7.
[46] Jégourel D, Delépée R, Breton F, Rolland A, Vidal R, Agrofoglio LA. Molecularly imprinted polymer of 5-methyluridine for solid-phase extraction of pyrimidine nucleoside cancer markers in urine. Bioorg Med Chem 2008;16:8932–9.
[47] Kempe M. Antibody-mimicking polymers as chiral stationary phases in HPLC. Anal Chem 1996;68:1948–53.
[48] Rachkov A, Minoura NJ. Recognition of oxytocin and oxytocin-related peptides in aqueous media using a molecularly imprinted polymer synthesized by the epitope approach. J Chromatogr A 2000;889:111–8.
[49] Vaidya AA, Lele BS, Kulkami MG, Mashelkar RA. Creating a macromolecular receptor by affinity imprinting. J Appl Polym Sci 2001;81:1075–83.
[50] Giraudi G, Giovannoli C, Tozzi C, Baggiani C, Anfossi L. Estradiol binding synthetic polypeptides. Chem Commun 1999;13:1135–6.
[51] Giraudi G, Giovannoli C, Tozzi C, Baggiani C, Anfossi L. Molecular recognition properties of peptide mixtures obtained by polymerisation of amino acids in the presence of estradiol. Anal Chim Acta 2003;481:41–53.
[52] Chianella I, Lotierzo M, Piletsky S, Tothill IE, Chen B, Turner APF. Rational design of a polymer specific for microcystin-LR using a computational approach. Anal Chem 2002;74:1288–93.
[53] Chianella I, Piletsky SA, Tothill IE, Chen B, Turner APF. Combination of solid phase extraction cartridges and MIP-based sensor for detection of microcystin-LR. Biosens Bioelectron 2003;18:119–27.
[54] Chianella I, Karim K, Piletska EV, Preston C, Piletsky SA. Computational design and synthesis of molecularly imprinted polymers with high binding capacity for pharmaceutical applications-model case: Adsorbent for abacavir. Anal Chim Acta 2006;559:73–8.

[55] Lotierzo M, Henry OYF, Piletsky S, Tothill I, Cullen D, Kania M, et al. Surface plasmon resonance sensor for domoic acid based on grafted imprinted polymer. Biosens Bioelectron 2004;20:145–52.
[56] Tothill IE, Turner APF. Biosensors. In: Caballero B, Trugo L, Finglas P, editors. Encyclopaedia of food sciences and nutrition. 2nd ed. Academic Press; 2003. ISBN: 0-12-227055-X.
[57] Haab BB. Antibody arrays in cancer research. Mol Cell Proteomics 2005;4:377–83.
[58] Lin J, Ju H. Electrochemical and chemiluminescent immunosensors for tumor markers. Biosens Bioelectron 2005;20:1461–70.
[59] Wang J. Electrochemical biosensors: towards point of care cancer diagnostics. Biosens Bioelectron 2006;21:1887–92.
[60] Wang J, Kawde A. Pencil-based renewable biosensor for label-free electrochemical detection of DNA hybridization. Anal Chim Acta 2000;431:219–25.
[61] Tansil NC, Xie F, Xie H, Gao Z. An ultrasensitive nucleic acid biosensor based on the catalytic oxidation of guanine by a novel redox threading intercalator. Chem Commun 2005;8:1064–6.
[62] Zhu Q, Chai YQ, Yuan R, Wang N, Li XL. Development of a biosensor for the detection of carcinoembryonic antigen using Faradic impedance spectroscopy. Chem Lett 2005;34:1682–3.
[63] O'Sullivan CK, Guilbault GG. Commercial quartz crystal microbalances—theory and applications. Biosens Bioelectron 1999;14:663–70.
[64] Zhou XC, Huang LQ, Li SFY. Microgravimetric DNA sensor based on quartz crystal microbalance: comparison of oligonucleotide immobilization methods and the application in genetic diagnosis. Biosens Bioelectron 2001;16:85–95.
[65] Zhou XD, Liu LJ, Hu M, Wang LL, Hu JM. Detection of hepatitis B virus by piezoelectric biosensor. J Pharm Biomed Anal 2002;27:341–5.
[66] Uludag Y, Tothill IE. Real-time detection of prostate specific antigen (PSA) in human serum using a fully automated QCM biosensor. Anal Chem Acta; submitted for publication.
[67] Chou SF, Hsu WL, Hwang JM, Chen CY. Development of an immunosensor for human ferritin, a nonspecific tumor marker, based on a quartz crystal microbalance. Anal Chim Acta 2002;453(2):181–9.
[68] Zhang B, Mao QG, Zhang X, Jiang TL, Chen M, Yu F, et al. A novel piezoelectric quartz micro-array immunosensor based on self-assembled monolayer for determination of human chorionic gonadotropin. Biosens Bioelectron 2004;19:711–20.
[69] Hansen KM, Thundat T. Microcantilever biosensors. Methods 2005;37: 57–64.
[70] Fu ZF, Liu H, Ju HX. Flow-through multianalyte chemiluminescent immunosensing system with designed substrate zone-resolved technique for sequential detection of tumor markers. Anal Chem 2006;79:6999–7005.
[71] Wilson MS. Electrochemical immunosensors for the simultaneous detection of two tumor markers. Anal Chem 2005;77:1496–502.
[72] Wilson MS, Nie W. Multiplex measurement of seven tumor markers using an electrochemical protein chip. Anal Chem 2006;78:6476–83.
[73] Willner I, Baron R, Willner B. Integrated nanoparticle–biomolecule systems for biosensing and bioelectronics. Biosens Bioelectron 2007;22:1841–52.
[74] Katz E, Willner I, Wang J. Electroanalytical and bioelectroanalytical systems based on metal and semiconductive nanoparticles. Electroanalysis 2004;16:19–44.
[75] Jain KK. Applications of biochips: from diagnostics to personalized medicine. Curr Opin Drug Discov Dev 2004;7:285–9.
[76] Sun CP, Liao JC, Zhang YH, Gau V, Mastali M, Babbitt JT, et al. Rapid, species-specific detection of uropathogen 16S rDNA and rRNA at ambient temperature by dot-blot hybridization and an electrochemical sensor array. Mol Genet Metab 2005;84:90–9.
[77] Wang WU, Chen C, Lin KH, Fang Y, Lieber CM. Labelfree detection of small-molecule–protein interactions by using nanowire nanosensors. Proc Natl Acad Sci USA 2005;102:3208–12.
[78] Woolley AT, Guillemette C, Cheung CL, Housman DE, Lieber CM. Direct haplotyping of kilobase-size DNA using carbon nanotube probes. Nat Biotechnol 2000;18:760–3.
[79] Baller MK, Lang HP, Fritz J, Gerber C, Gimzewsk JK, Drechsler U, et al. A cantilever array-based artificial nose. Ultramicroscopy 2000;82:1–9.
[80] Wu G, Datar RH, Hansen KM, Thundat T, Cote RJ, Majumdar A. Bioassay of prostate-specific antigen (PSA) using microcantilevers. Nat Biotechnol 2001;19:856–60.
[81] Maxwell DJ, Taylor JR, Nie SM. Self-assembled nanoparticle probes for recognition and detection of biomolecules. J Am Chem Soc 2002;124:9606–12.
[82] Gerion D, Pinaud F, Williams SC, Parak WJ, Zanchet D, Weiss S, et al. Synthesis and properties of biocompatible watersoluble silica-coated CdSe/ZnS semiconductor quantum dots. J Phys Chem B 2001;105:8861–71.
[83] Gerion D, Parak WJ, Williams SC, Zanchet D, Micheel CM, Alivisatos AP. Sorting fluorescent nanocrystals with DNA. J Am Chem Soc 2002;124:7070–4.
[84] Kim S, Lim YT, Soltesz EG, De Grand AM, Lee J, Nakayama A, et al. Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping. Nat Biotechnol 2004;22:93–7.
[85] Choi J-W, Kang D-Y, Jang Y-H, Kim H-H, Min J, Oh B-K. Ultra sensitive surface plasmon resonance based immunosensor for prostate specific antigen using gold nanoparticale antibody complex. Colloids Surf A: Physicochem Eng Aspects 2008;313–314:655–9.
[86] Wang J. Nanomaterial-based electrochemical biosensors. Analyst 2005;130: 421–6.
[87] Logrieco A, Arrigan DWM, Brengel-Pesce K, Siciliano P, Tothill IE. Microsystems technology solutions for rapid detection of toxigenic fungi and mycotoxins. J Food Addit Contam 2005;22(4):335–44.
[88] Katz E, Willner I. Biomolecular-functionalized carbon nanotubes: applications in nanobioelectronics. Chem Phys Chem 2004;5:1085–91.
[89] Zheng G, Patolsky F, Cui Y, Wang WU, Lieber CM. Multiplexed electrical detection of cancer markers with nanowire sensor arrays. Nat Biotechnol 2005;23:1294–301.
[90] Okuno J, Maehashi K, Kerman K, Takamura Y, Matsumoto K, Tamiya E. Label-free immunosensor for prostate-specific antigen based on single walled carbon nanotube array-modified microelectrodes. Biosens Bioelectron 2007;22:2377–81.
[91] Abrantes M, Magone MT, Boyd LF, Schuck P. Adaptation of a surface plasmon resonance biosensor with microfluidics for use with small sample volumes and long contact times. Anal Chem 2001;73:2828–35.
[92] Wang PC, Gao J, Lee CS. High-resolution chiral separation using microfluidics-based membrane chromatography. J Chromatogr A 2002;942:115–22.
[93] Kurita R, Hayashi K, Horiuchi T, Niwa O, Maeyama K, Tanizawa K. Differential measurement with a microfluidic device for the highly selective continuous measurement of histamine released from rat basophilic leukemia cells (RBL-2H3). Lab Chip 2002;2:34–8.
[94] Furdui VI, Harrison DJ. Immunomagnetic T cell capture from blood for PCR analysis using microfluidic systems. Lab Chip 2004;4:614–8.
[95] Kojima K, Hiratsuka A, Suzuki H, Yano K, Ikebukuro K, Karube I. Electrochemical protein chip with arrayed immunosensors with antibodies immobilized in a plasma-polymerized film. Anal Chem 2003;75:1116–22.
[96] Guan JG, Miao YQ, Chen JR. Prussian blue modified amperometric FIA biosensor: one-step immunoassay for alpha-fetoprotein. Biosens Bioelectron 2004;19:789–94.
[97] Liang RP, Qiu JD, Zou XY, Cai PX. Breast cancer antigen immunosensor based on the functional sol–gel film. Chem J Chin Univ 2004;25:425.
[98] He Z, Gao N, Jin W. Determination of tumor marker CA125 by capillary electrophoretic enzyme immunoassay with electrochemical detection. Anal Chem Acta 2003;497:75–81.
[99] Dai Z, Yan F, Chen J, Ju H. Reagentless amperometric immunosensors based on direct electrochemistry of horseradish peroxidase for rapid determination of carcinoma antigen-125. Anal Chem 2003;75:5429–34.
[100] Du D, Yan F, Liu SL, Ju HX. Immunological assay for carbohydrate antigen 19-9 using an electrochemical immunosensor and antigen immobilization in titania sol–gel matrix. J Immunol Methods 2003;283:67–75.
[101] Dai Z, Chen J, Yan F, Ju H. Electrochemical sensor for immunoassay of carcinoembryonic antigen based on thionine monolayer modified gold electrode. Cancer Detect Prev 2005;29:233–40.
[102] Tan F, Yan F, Ju HX. A designer ormosil gel for preparation of sensitive immunosensor for carcinoembryonic antigen based on simple direct electron transfer. Electrochem Commun 2006;8:1835–9.
[103] Lin JH, Yan F, Ju HX. Simple noncompetitive enzyme immunoassay for carcinoembryonic antigen by flow injection chemiluminescence. Clin Chim Acta 2004;341:109–15.
[104] Chou S-F, Hsu WL, Hwang JM, Chen CY. Development of an immunosensor for human ferritin, a nonspecific tumor marker, based on surface plasmon resonance. Biosens Bioelectron 2004;19:999–1005.
[105] Nakamura N, Lim TK, Jeong JM, Matsunaga T. Flow immunoassay for detection of human chorionic gonadotrophin using a cation exchange resin packed capillary column. Anal Chim Acta 2001;439:125–30.
[106] Meyerhoff ME, Duan C, Meusel M. Novel non-separation sandwich-type electrochemical enzyme immunoassay system for detecting marker proteins in undiluted blood. Clin Chem 1995;41:1378–84.
[107] Sarkar P, Pal PS, Ghosh D, Setford SJ, Tothill IE. Amperometric biosensors for detection of the prostate cancer marker (PSA). Int J Pharm 2002;238:1–9.
[108] Fernandez-Sanchez C, McNeil CJ, Rawson K, Nilsson O. Disposable noncompetitive immunosensor for free and total prostate-specific antigen based on capacitance measurement. Anal Chem 2004;76:5649–56.
[109] Wee KW, Kang GY, Park J, Kang J, Yoon Y, Park DS, et al. Novel electrical detection of label-free disease marker proteins using piezoresistive self-sensing microcantilevers. Biosens Bioelectron 2005;20:1932–8.
[110] Besselink GAJ, Kooyman RPH, van Os PJHJ, Engbers GHM, Schasfoort RBM. Signal amplification on planar and gel-type sensor surfaces in surface plasmon resonance-based detection of prostate-specific antigen. Anal Biochem 2004;333:165–73.
[111] Rasooly A, Jacobson J. Development of biosensors for cancer clinical testing. Biosens Bioelectron 2005;21:1851–8.